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Fetal-maternal nitrite exchange in sheep: Experimental data, a computational model and an estimate of placental nitrite permeability

Hobe J. Schroeder [b], Eriko Kanda [a], Gordon G. Power [b], Arlin B. Blood [a, b, *]

[a] Department of Pediatrics, Division of Neonatology, Loma Linda University School of Medicine, Loma Linda, CA, USA
[b] Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA

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ABSTRACT

Introduction: Nitrite conveys NO-bioactivity that may contribute to the high-flow, low-resistance character of the fetal circulation. Fetal blood nitrite concentrations depend partly on placental permeability which has not been determined experimentally. We aimed to extract the placental permeability-surface (PS) product for nitrite in sheep from a computational model.

Methods: An eight-compartment computational model of the fetal–maternal unit was constructed (Matlab® (R2013b (8.2.0.701), MathWorks Inc., Natick, MA). Taking into account fetal and maternal body weights, four variables (PS, the rate of nitrite metabolism within red cells, and two nitrite distribution volumes, one with and one without nitrite metabolism), were varied to obtain optimal fits to the experimental plasma nitrite profiles observed following the infusion of nitrite into either the fetus ($n = 7$) or the ewe ($n = 8$).

Results: The model was able to replicate the average and individual nitrite–time profiles ($r^2 > 0.93$) following both fetal and maternal nitrite infusions with reasonable variation of the four fitting parameters. Simulated transplacental nitrite fluxes were able to predict umbilical arterial-venous nitrite concentration differences that agreed with experimental values. The predicted PS values for a 3 kg sheep fetus were 0.024 ± 0.005 l·min^{-1} in the fetal–maternal direction and 0.025 ± 0.003 l·min^{-1} in the maternal–fetal direction (mean ± SEM). These values are many-fold higher than the reported PS product for chloride anions across the sheep placenta.

Conclusion: The result suggests a transfer of nitrite across the sheep placenta that is not exclusively by simple diffusion through water-filled channels.

1. Introduction

Nitric oxide (NO) is a potent vasodilating agent that is generated in vivo by NO synthases (NOS) in endothelial [1] and other cells. NO itself is short lived, but related compounds such as nitrite (NO_2^-) and nitrosothiols carry NO-like bioactivity throughout the body [2]. Among the many pathways that interconvert these species are those mediated by nitrite reductase proteins such as members of the heme-containing globin superfamily. These reactions are favored when O_2 concentrations are low [3], constituting an hypoxia-dependent mode of NO production. Thus, for the mammalian fetus in utero, where average oxyhemoglobin saturation is far less than in the adult, nitrite as a source of NO bioactivity becomes of particular interest and may account in part for the vasodilated state of the fetal circulation [4,5].

Fetal plasma nitrite levels are in the range of 200–500 nM [5,6] and likely to depend on placental transfer of nitrite from mother to fetus, as well as in the reverse direction. Evidence for placental transfer is shown by fetal levels closely following maternal levels [5], and then falling about 50% immediately after birth [7]. Placental permeability of nitrite has not yet been determined experimentally, and such measurements would be difficult to interpret because nitrite is metabolized quickly in blood [8], moves rapidly between body fluid compartments [9], is produced endogenously from NO [10], and may be metabolized in placental tissues.

We therefore decided to build a computational model of fetal–maternal nitrite exchange based on experiments in chronically instrumented fetal sheep. In these experiments, nitrite was infused intravenously to either the fetus or the ewe, and the time

* Corresponding author. Department of Pediatrics, Loma Linda University, 11175 Campus St, 11121 Coleman, Loma Linda, CA 92354, USA.
E-mail address: ablood@llu.edu (A.B. Blood).

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Glossary

NO	nitric oxide
L-NAME	L-N^G-Nitroarginine methyl ester
PS	permeability-surface product
RBC	red blood cell
BW	body weight
PV	plasma volume
BV	blood volume
HNO_2	nitrous acid
HCl	hydrochloric acid

courses of plasma nitrite concentrations in both circulations were measured, as were uterine and umbilical flow rates and other physiological variables. The nitrite concentration profile is then partly dependent on placental nitrite permeability, and we sought to separate this parameter from other factors using the model, and to gain insight into the distribution of nitrite in various body compartments.

2. Materials and methods

2.1. Animal experiments

The animal protocol was pre-approved by the Loma Linda University Institutional Animal Care and Use Committee. Anesthetized ewes and their fetuses (gestational age 127 to 131, term 145 days) were surgically instrumented with femoral arterial and venous catheters, with catheters in a uterine vein and an umbilical vein, and with Transonic® flow probes around one uterine artery and the intra-abdominal common umbilical vein. The instrumentation allowed for intravenous infusion of sodium nitrite into either a maternal or fetal femoral vein, the withdrawal of blood samples after selected time intervals (≥5 min) for the measurement of plasma nitrite concentrations in arteries as well as in the umbilical and uterine veins, for the determination of arterial blood parameters (pH, PO_2, PCO_2, percentage of hemoglobin oxygen saturation, percentage methemoglobin, hemoglobin concentration) and the continuous measurement of uterine and intra-abdominal umbilical vein blood flow rates, blood pressures, and heart rates. In the fetus, intra-abdominal umbilical venous flow is identical with fetal placental flow whereas in the ewe maternal placental flow is mirrored only partially by the uterine flow measured at one side, and where the latter also supplies not only placental tissues (cotyledons) but also the myo- and endometrium. Thus, our experimental data are based on the assumption of equal division of flow between the two uterine horns and negligible net nitrite exchange with the myo- and endometrium, which comprise ~10% of uterine blood flow [11]. The duration of an experiment was 200 min. During this time, flow rates and hemoglobin oxygen saturations remained relatively stable, and thus their time-averaged values were used in this study.

Three days after instrumentation, sodium nitrite was infused either into a fetal (7 animals) or a maternal (8 animals) femoral vein (cf. Fig. S1 in Supplement). The mode of infusion was identical in both groups: at time zero, a saline bolus (6 ml) containing sodium nitrite (12 $mmol \cdot l^{-1}$ in fetuses) was injected within about 6 s, followed immediately by a continuous infusion (1 $ml \cdot min^{-1}$) for 60 min. Then the bolus injection was repeated, and the nitrite infusion was continued at twice the initial rate (by doubling the nitrite concentration) for another 60 min when the infusion was stopped. Data were collected until 200 min after the start of the nitrite infusion.

The two groups differed in the amount of nitrite infused. With fetal application, the amount of nitrite infused was the same for each animal, which was 72 μmoles for the boluses and 4.2 and 8.4 $\mu mol \cdot min^{-1}$ (concentrations: 4.2 and 8.4 $mmol \cdot l^{-1}$, respectively) for the first and second infusion rates, respectively, a total of 900 μmoles. For maternal application, the amount of nitrite depended on maternal body weight (BW): each bolus transferred 3.6 $\mu mol \cdot kg^{-1}$ BW into the ewe, and infusion rates were 0.121 and 0.242 $\mu mol\ min^{-1} \cdot kg^{-1}$ BW, respectively. On average, about 1500 μmoles were infused.

2.2. Blood sample assays

Average arterial blood gas values, corrected to maternal and fetal body temperatures, were measured at experimental time = 120 min using an ABL 800 blood gas analyzer (Radiometer, Copenhagen), with PO_2 and PCO_2 reported in mmHg and bicarbonate reported in mmol/l. During the fetal nitrite infusion protocol, maternal arterial levels were as follow: pH 7.44 ± 0.01, PO_2 109.9 ± 2.0 mmHg, PCO_2 33.6 ± 1.0 mmHg, bicarbonate 22.6 ± 0.9 mM. Fetal arterial levels were as follow: pH 7.31 ± 0.01, PO_2 20.0 ± 1.5 mmHg, PCO_2 47.2 ± 0.7 mmHg, bicarbonate 23.0 ± 0.8 mM. During the maternal nitrite infusion protocol, maternal arterial levels were as follow: pH 7.46 ± 0.01, PO_2 108.4 ± 2.7 mmHg, PCO_2 34.1 ± 0.49 mmHg, bicarbonate 23.0 ± 0.9 mM. Fetal arterial levels were as follow: pH 7.33 ± 0.01, PO_2 22.4 ± 1.7 mmHg, PCO_2 47.7 ± 1.4 mmHg, bicarbonate 23.6 ± 0.9 mM. These values are within the normal range for the chronic fetal sheep animal preparation.

Blood samples for plasma nitrite measurement were centrifuged for 30 s at 12,000 rpm immediately after collection. Plasma was stored at < 80 °C until assay. Nitrite was measured using the triiodide chemiluminescence method as previously described [12,13]. It should be noted that the method measures the combined concentrations of nitrite, S-nitrosothiols, N-nitrotyrosines and iron-nitrosyl species [14,15] above 10 nM and has a precision of ±5 nM. In our hands, removal of nitrite with acid sulfanilamide, as previously described [14,15], eliminates >97% of the signal in samples collected from either maternal or fetal sheep (data not shown), and thus we designate these measurements here as nitrite. The assay does not detect nitrate.

2.3. The computational model

For model construction, the Simbiology® application of Matlab® (R2013b (8.2.0.701), MathWorks Inc., Natick, MA) was used. The program allows the build-up of a model structure and its parameters, and it then generates the necessary differential equations. The model structure is illustrated in Fig. 1 in a simplified form. A detailed version representing experiments with fetal or maternal nitrite application is available for download from the Placenta website as MatLab® *.sbproj files and a supplemental document which lists all model equations.

Because nitrite was administered to either the fetus or the ewe, a fetal and a maternal infusion model were used, both of identical structure but with a different set of parameter values.

In the model, nitrite can either move between compartments, or it can be irreversibly “converted” and thus removed permanently from the system, as in red cells, for example. Compartments are the maternal and fetal blood volumes that are connected by placental nitrite exchange, and two different distribution volumes for nitrite, one of which is “passive” (A), and the other is “active” (B) in that it is able to convert nitrite.

Nitrite conversion in the red blood cell (RBC) compartment (see below) is controlled by the parameter Kex* (* indicates either fetal